Applicants hereby submit a marked up version of Replacement Pages with markings to show changes made.

cause the reversal of particle formation may be, but not limited to, the pH, ionic strength, oxidative or reductive conditions or agents, or enzymatic activity.

DNA Template Polymerization

Low molecular weight cations with valency < +3 fail to condense DNA in aqueous solutions under normal conditions. However, cationic molecules with the charge <+3 can be polymerized in the presence of DNA and the resulting polymers can cause DNA to condense into compact structures. Such an approach is known in synthetic polymer chemistry as template polymerization. During this process, monomers (which are initially weakly associated with the template) are positioned along template's backbone, thereby promoting their polymerization. Weak elecrostatic association of the nascent polymer and the template becomes stronger with chain growth of the polymer. Trubetskoy et al used two types of polymerization reactions to achieve DNA condensation: step polymerization and chain polymerization (VS Trubetskoy, VG Budker, LJ Hanson, PM Slattum, JA Wolff, LE Hagstrom. Nucleic Acids Res. 26:4178-4185, 1998) U.S. 08/778,657, U.S. 09/000,692, U.S. 97/24089, U.S. 09/070299, and U.S. 09/464,871. Bis(2-aminoethyl)-1,3-propanediamine (AEPD), a tetraamine with 2.5 positive charges per molecule at pH 8 was polymerized in the presence of plasmid DNA using cleavable disulfide amino-reactive cross-linkers dithiobis (succinimidyl propionate) and dimethyl-3,3'-dithiobispropionimidate. Both reactions yielded DNA/polymer complexes with significant retardation in agarose electrophoresis gels demonstrating significant binding and DNA condensation. Treatment of the polymerized complexes with 100 mM dithiothreitol (DTT) resulted in the pDNA returning to its normal supercoiled position following electrophoresis proving thus cleavage the backbone of the. The template dependent polymerization process was also tested using a 14 mer peptide encoding the nuclear localizing signal (NLS) of SV40 T antigen SEQ ID NO: 1 (CGYGPKKKRKVGGC) as a cationic "macromonomer". Other studies included pegylated comonomer (PEG-AEPD) into the reaction mixture and resulted in "worm"-like structures (as judged by transmission electron microscopy) that have previously been observed with DNA complexes formed from block co-polymers of polylysine and PEG (MA Wolfert, EH Schacht, V Toncheva, K Ulbrich, O Nazarova, LW Seymour Human Gene Ther. 7:2123-2133, 1996). Blessing et al used bisthiol derivative of spermine and reaction of thiol-disulfide exchange to promote chain growth. The presence of DNA accelerated the polymerization reaction as measured the rate of disappearance of free thiols in the reaction mixture (T Blessing, JS Remy, JP Behr. J. Am. Chem. Soc. 120:8519-8520, 1998).

Salt

A salt is any compound containing ionic bonds, that is bonds in which one or more electrons are transferred completely from one atom to another.

Interpolyelectrolyte Complexes

An interpolyelectrolyte complexe is a noncovalent interaction between polyelectrolytes of opposite charge.

Charge, Polarity, and Sign

The charge, polarity, or sign of a compound refers to whether or not a compound has lost one or more electrons (positive charge, polarity, or sign) or gained one or more electrons (negative charge, polarity, or sign).

Cell Targeting Signals

Cell targeting signal (or abbreviated as the Signal) is defined in this specification as a molecule that modifies a biologically active compounds such as drug or nucleic acid and can direct it to a cell location (such as tissue) or location in a cell (such as the nucleus) either in culture or in a whole organism. By modifying the cellular or tissue location of the foreign gene, the function of the biologically active compound can be enhanced.

The cell targeting signal can be a protein, peptide, lipid, steroid, sugar, carbohydrate, (non-expresssing) polynucleic acid or synthetic compound. The cell targeting signal enhances cellular binding to receptors, cytoplasmic transport to the nucleus and nuclear entry or release from endosomes or other intracellular vesicles.

Nuclear localizing signals enhance the targeting of the pharmaceutical into proximity of the nucleus and/or its entry into the nucleus. Such nuclear transport signals can be a protein or a peptide such as the SV40 large T ag NLS or the nucleoplasmin NLS. These nuclear localizing signals interact with a variety of nuclear transport factors such as the NLS receptor (karyopherin alpha) which then interacts with karyopherin beta. The nuclear transport proteins themselves could also function as NLS's since they are targeted to the nuclear pore and nucleus. For example, karyopherin beta itself could target the DNA to the nuclear pore complex. Several peptides have been derived from the SV40 T antigen. These include a short NLS SEQ ID NO: 2 (H-CGYGPKKKRKVGG-OH) or long NLS's SEQ ID NOs: 3 & 4 (H-CKKKSSSDDEATADSQHSTPPKKKRKVEDPKDFPSELLS-OH and H-CKKKWDDEATADSQHSTPPKKKRKVEDPKDFPSELLS-OH). Other NLS peptides have

been derived from M9 protein SEQ ID NO: 5 (CYNDFGNYNNQSSNFGPMKQGNFGGRSSGPY), E1A SEQ ID NO: 6 (H-CKRGPKRPRP-OH), nucleoplasmin SEQ ID NO: 7 (H-CKKAVKRPAATKKAGQAKKKKL-OH),and c-myc SEQ ID NO: 8 (H-CKKKGPAAKRVKLD-OH).

Signals that enhance release from intracellular compartments (releasing signals) can cause DNA release from intracellular compartments such as endosomes (early and late), lysosomes, phagosomes, vesicle, endoplasmic reticulum, golgi apparatus, trans golgi network (TGN), and sarcoplasmic reticulum. Release includes movement out of an intracellular compartment into cytoplasm or into an organelle such as the nucleus. Releasing signals include chemicals such as chloroquine, bafilomycin or Brefeldin A1 and the ER-retaining signal (KDEL sequence), viral components such as influenza virus hemagglutinin subunit HA-2 peptides and other types of amphipathic peptides.

Cellular receptor signals are any signal that enhances the association of the biologically active compound with a cell. This can be accomplished by either increasing the binding of the compound to the cell surface and/or its association with an intracellular compartment, for example: ligands that enhance endocytosis by enhancing binding the cell surface. This includes agents that target to the asialoglycoprotein receptor by using asiologlycoproteins or galactose residues. Other proteins such as insulin, EGF, or transferrin can be used for targeting. Peptides that include the RGD sequence can be used to target many cells. Chemical groups that react with thiol, sulfhydryl, or disulfide groups on cells can also be used to target many types of cells. Folate and other vitamins can also be used for targeting. Other targeting groups include molecules that interact with membranes such as lipids, fatty acids, cholesterol, dansyl compounds, and amphotericin derivatives. In addition viral proteins could be used to bind cells.

Interaction Modifiers

An interaction modifier changes the way that a molecule interacts with itself or other molecules, relative to molecule containing no interaction modifier. The result of this modification is that self-interactions or interactions with other molecules are either increased or decreased. For example cell targeting signals are interaction modifiers that change the interaction between a molecule and a cell or cellular component. Polyethylene glycol is an interaction modifier that decreases interactions between molecules and themselves and with other molecules.

demonstrated by its ability to release heme from red blood cells (hemolysis). In addition, dimethylmaleamic-modified mellitin (DM-Mel) reverts to melittin in the acidic environment of the endosome causes endosomal release as seen by the diffuse staining of fluorescein-labled dextran in our endosomal release assay.

More specifically membrane active compounds allow for the transport of molecules with molecular weight greater than 50 atomic mass units to cross a membrane. This transport may be accomplished by either the total loss of membrane structure, the formation of holes (or pores) in the membrane structure, or the assisted transport of compound through the membrane. In addition, transport between liposomes, or cell membranes, may be accomplished by the fusion of the two membranes and thereby the mixing of the contents of the two membranes.

Membrane active peptides.

Membrane active peptides are peptides that have membrane activity. There are many naturally occurring membrane active peptides such as cecropin (insects), magainin, CPF 1, PGLa, Bombinin BLP-1 (all three from amphibians), melittin (bees), seminalplasmin (bovine), indolicidin, bactenecin (both from bovine neutrophils), tachyplesin 1 (crabs), protegrin (porcine leukocytes), and defensins (from human, rabbit, bovine, fungi, and plants). Gramicidin A and gramicidin S (bacillus brevis), the lantibiotics such as nisin (lactococcus lactis), androctonin (scorpion), cardiotoxin I (cobra), caerin (frog litoria splendida), dermaseptin (frog). Viral peptides have also been shown to have membrane activity, examples include hemagglutinin subunit HA-2 (influenza virus), E1 (Semliki forest virus), F1 (Sendai and measles viruses), gp41 (HIV), gp32 (SIV), and vp1 (Rhino, polio, and coxsackie viruses). In addition synthetic peptides have also been shown to have membrane activity. Synthetic peptides that are rich in leucines and lysines (KL or KL_n motif) have been shown to have membrane activity. In particular, the peptide SEQ ID NO: 9 H_2N-KLLKLLLKLWLKLLKLLLKLL-CO_2, termed KL_3, is membrane active.

Polymers

A polymer is a molecule built up by repetitive bonding together of smaller units called monomers. In this application the term polymer includes both oligomers which have two to about 80 monomers and polymers having more than 80 monomers. The polymer can be linear, branched network, star, comb, or ladder types of polymer. The polymer can be a